IMPACT OF VIRAL INFECTION ON ABSORPTION AND SCATTERING PROPERTIES OF MARINE BACTERIA AND PHYTOPLANKTON

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LONG-TERM GOALS

The long-range goal of our research was to document the role of viruses in light scattering in the sea. The DEPSCoR project was a joint venture between the University of New England and its affiliate, Bigelow Laboratory for Ocean Sciences.

OBJECTIVES

The objectives of this work were to: 1) define the typical time scale for the shift in inherent optical properties (a, b, c, and bb) associated with viral infection of marine bacteria (as compared to non infected control populations), 2) define the maximum possible rates of change of inherent optical properties (a, b, c, and b_b) under conditions of high virus multiplicity of infection, and 3) define the concurrent changes in 0.03-100 μm size spectra associated with viral infection of marine bacteria.

APPROACH

This work involved a significant number of laboratory experiments leading to mesocosm experiments off the Bigelow Dock (West Boothbay Harbor, ME). Our experimental design assured that 1) inoculated viruses were host specific, 2) host culturing conditions were optimal for virus assays, 3) host growth conditions were sufficiently understood from preliminary experiments in aqueous media, 4) initial viral concentrations were easily set, 5) bottle effects were minimized because of the large volumes, and 6) mesocosms provided a realistic scale for observing viral infection dynamics in blooms of marine organisms. In our review of the literature, we have observed many cases where strict controls were not run such that results were too ambiguous to interpret.

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WORK COMPLETED

Isolation of Heterotrophic Marine Bacteria and their Bacteriophage
Candidate marine host bacteria were isolated from Saco Bay, the Saco River (high tide) and West Boothbay Harbor using a membrane filtration method (Balch et. al. 2000). An extensive effort, involving an exhaustive number of "wet chemistry" methods, was made to identify the specific hosts of bacterial viruses used during the first year of the study. In all, nine of the most often used bacterial strains were identified.

Isolation of Viruses of Photosynthetic Marine Bacteria
Methods for the isolation of viruses active against species of the genus Synechococcus involved the use of axenic host cultures from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), including 4 strains plus water samples collected from West Boothbay Harbor. Our procedure was a modification of the enrichment method for isolation of viruses of heterotrophic bacteria (Balch et al. 2000). Eventually, a viral pathogen for each of the above host strains was isolated and purified.

Isolation of Viruses of the Marine Alga Emaliania huxleyi
Preliminary studies were conducted to isolate viruses infecting the coccolithophore *E. huxleyi*. The method was a modification of the enrichment technique described previously. In each case, host populations were reduced to unobservable levels, with a corresponding clarification of test flasks to a "gin clear" quality save for some large aggregates at the bottom of the flask. Subsequent inoculation of new host cultures with lysate material resulted in a similar clearing within 10-12 days.

Concentration, Purification, Enumeration and Electron Microscopy of Virus Isolates.
Concentration /purification methods for all virus types were similar to those described in Balch et. al. (2000). Virus enumeration methods used depended on the host type. Assays for viruses of heterotrophic marine bacteria typically involved the use of plaque assays (Adams 1959) and fluorescence staining with SYBR green (Noble and Fuhrman, 1998; Balch et. al. 2000). Viruses of photosynthetic marine bacteria were assayed using SYBR green staining, or a modified Most Probable Number (MPN) method. *Cyanophage Host*

Host Range Studies
Viruses, originally isolated from West Boothbay Harbor on three strains of Synechococcus, were tested on a variety of CCMP Synechococcus strains representing a number of geographical locales, to determine whether cross reactivity occurred among these diverse species. In addition to the host strains listed above, viruses were also tested against five other CCMP strains. Results indicated no cross-reactivity between the viruses and any of the original host species These date suggest that despite host strain variations (growth conditions, pigment production, genetic variations), they retained sufficient similar characteristics (e.g. virus attachment sites, synthetic machinery) to permit infection and replication by several prototype viruses.

Virus Growth Characteristics
We have determined the growth characteristics of all of the marine virus isolates described above. Studies with viruses of heterotrophic bacteria have included short-term (< 24 hr) and intermediate experiments (48-72 hr), as well as weeklong chemostat studies. Six of the nine new phage isolates demonstrated strong growth characteristics, with 2 to 3 log increases in titer over the course of each growth experiment. Average burst time (i.e. time for a single virus replication cycle) was approximately 60 min. Complete clarification of host cultures occurred in less than 24 hr. Viruses of photosynthetic bacteria, on the other hand, replicated over a significantly longer period. On average, some 3-6 days were

required for a single replication cycle. Culture clarification required 6-8 days. Studies to date with the coccolithophore virus indicate an even longer growth cycle (10-14 days for culture clarification).

Optical Measurements
A Dawn Laser Light Scattering Photometer (Wyatt Technologies, Santa Barbara, CA) was used for measuring the volume scattering function, and the calculation of the backscattering coefficient (b_b). This instrument measures the volume scattering function at 15 angles at a rate of 400 Hz and averages the data over any pre-set time period. All details of the Wyatt "Dawn" light scattering photometer can be found in Balch et al. (1999; see the legend to their table 2; Balch et al. 2002). Absorption measurements of suspensions of photosynthetic algae were made using a Camspec scanning spectrophotometer, equipped with integrating sphere to maximize the capture of forward-scattered light. Absorbance by algal suspensions was measured at one-nanometer resolution, between 200-800nm. Distilled water blanks were subtracted from all algal spectra in order to estimate particulate and dissolved absorbance. Spectra were further corrected for scattering losses by subtracting the un-corrected absorbance at 750nm (assuming insignificant absorbance by particulate and dissolved materials at this wavelength).

Infection Experiments
Continuous culture studies were performed to examine the kinetics of infection with one marine bacterium, strain LS-05, which will help understand the optical changes that we are observing. Infection experiments with heterotrophic bacteria, photosynthetic bacteria and eukaryotic algae were performed by adding diluted host bacteria to two sterile, borosilicate containers. Host was always derived from log phase cultures and maintained at their ambient growth conditions. Viruses were added to one of the containers using a multiplicity of infection (MOI) of 1.0, as determined by pre-experimental counts. At regular intervals, samples were withdrawn for optical measurements, host/virus enumeration (using plaque/plate counts, epi-fluorescence counts (for photosynthetic hosts) and Sybr-Green counts (Noble and Fuhrman 1998)) and FFFF analysis. Vials were then returned to incubation conditions until the next sampling. In all cases, viral infection caused a pronounced change in the light scattering properties of the marine bacterial and algal populations. Qualitatively, the turbidity of control and infected heterotrophic bacterial suspensions remained the same for several hours, after which infected samples were rapidly clarified. Relative to turbid controls, infected cultures were as clear as media blanks. Quantitatively, turbidity loss was observed as a sharp drop in the backscattering coefficients, attributable to two factors: 1) a distinct decrease in volume scattering values, and 2) for three host, a pronounced flattening of the VSF in the forward and backward directions. Accompanying these changes were simultaneous increases in virus concentrations in infected samples, and decreases in host bacteria after a short infection period. For photosynthetic algae (prokaryotes, or the eukaryote, *Emiliania huxleyi*), viral infection resulted in a strong loss in turbidity, backscattering, as well as absorption within the photosynthetic pigment absorption bands. Cell fluorescence dramatically decreased at the time of host lysis (Fig. 1). One difference between infection of algae versus heterotrophic bacteria was that algal infection resulted in more large aggregates following lysis (Fig. 2). We also noted that, as with heterotrophic bacteria hosts, following catastrophic lysis, there is evidence of re-growth of a virus-resistant strain (albeit, often at a slower growth rate).

IMPACT/APPLICATIONS

These are the first quantitative optical observations of phage-induced clarification of marine bacterial suspensions. While viruses themselves contribute little to backscattering, their infection and lysis of bacterioplankton can rapidly alter light scattering. Our observations

extend the understanding of the important role of viruses in the turnover of plankton populations (Fuhrman 1999) and the optical consequences of their infection. We hypothesize that viral infection of optically-active blooms provides an ideal mechanism for: 1) rapid reduction in turbidity due to profound changes in the VSFs of the particle suspension (as the scattering of the suspension changes from particle-scattering-dominated to molecular scattering-dominated), 2) changes in particle and dissolved absorption as cells release cDOM, and 3) aggregation of DOM into "Koike" particles"/ polymer-gels (Koike et al. 1990; Chin et al. 1998; Wells 1998), themselves a potentially important source of scattering (Stramski and Kiefer 1991).

TRANSITIONS

We have begun propagating several additional species of *Synechococcus* and other algal eucaryotes (viz. *E. huxleyi*), obtained from the Bigelow Provasoli-Guillard Center for the Culture of Marine Phytoplankton as well as field isolates. Pending additional funding, these could be used for understanding the role of viruses in the release of chromophoric dissolved organic carbon plus the subsequent changes to the optical properties of the sub-micron particles following infection as the cDOM forms micron-scale aggregates.

RELATED PROJECTS

This DEPSCoR work is a joint venture between the University of New England and its affiliate Bigelow Laboratory. Collaborative relationships have been maintained throughout the duration of this project with Dr. Ken Voss and Dr. Howard Gordon, both ONR-funded investigators at the University of Miami Dept. of Physics. This work is the logical outgrowth of a previous DEPSCoR project (N000149801999).

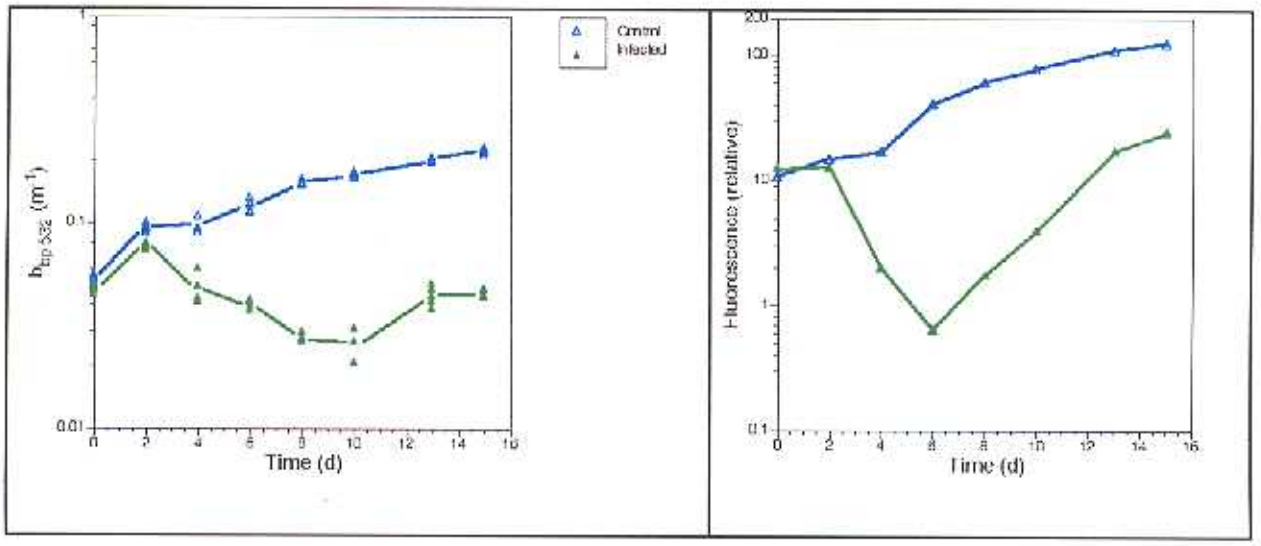

Fig. 1- A) Change in backscattering of a suspension of Emiliania huxleyi following virus infection. Blue, open triangles represent uninfected control cells while green, closed triangles represent infected cells. B) Fluorescence of control and infected cultures of E. huxleyi. Each time point in each panel has 5 separate measurements. In both panels, note that significant decreases in backscattering and chlorophyll fluorescence occurred following 2 days of infection. Also note the *increase* in backscattering after day 10, and fluorescence after day 6, in the infected flask, suggesting the growth of a viral-resistant strain of the host.

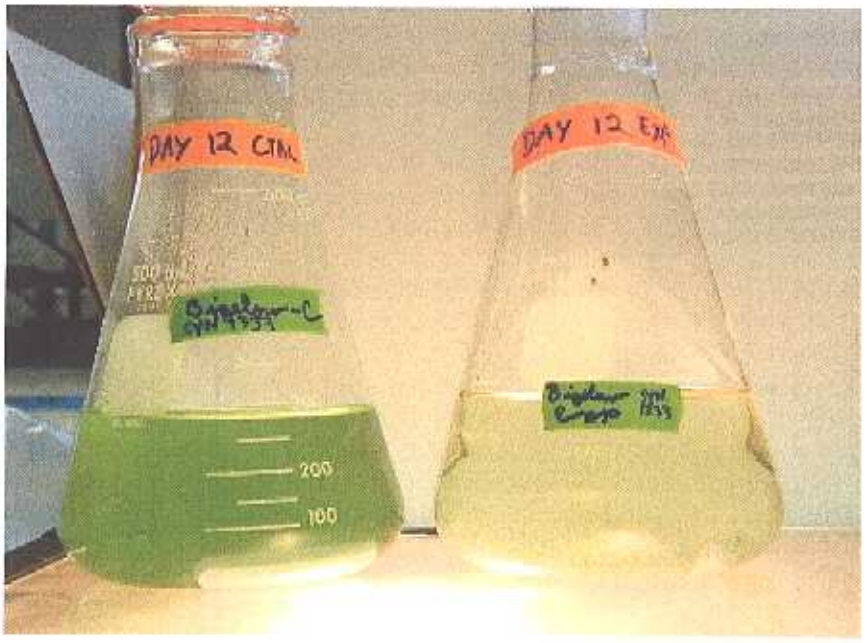

Figure 2-Control (left) and experimental (right) flasks of Synechococcus strain 1333, taken 12d after experimental flask was infected by purified Synechococcus virus. Flasks were illuminated from below to highlight the intense green reflectance of the uninfected control flask, and the greater transparency and loss of turbidity in the infected experimental flask. Note also the presence of aggregate particles in the experimental flask.

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